Sample Compositional Analysis

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Abstract

The ability to obtain an accurate chemical composition of biomass and biomass-derived samples using rapid and inexpensive methods is an important enabling technology for the commercialization of processes that convert biomass to bio-based materials, power, fuels and chemicals. New analytical methods are currently being developed at NREL that specifically address the needs of the emerging bioenergy industry. These new methods deal with compositional variability, an inherent characteristic of biomass feedstocks, by providing a means of monitoring the chemical composition of the feedstock and process intermediates in a time frame that allows real-time process adjustment and optimization

Our approach to reducing the time and cost of compositional analysis is the development of rapid analysis methods, where multivariate analysis techniques are used to extract chemical information from easily obtained spectroscopic data. The new Rapid Biomass Analysis methods reduce the cost of compositional analysis from $800 - $2,000 per sample to around $10 per sample. Rapid analysis methods provide results in minutes instead of days, time frame useful for process control. Rapid analysis methods often match the precision and accuracy of their calibration methods, so the advantages are obtained without loss of precision or accuracy.

In addition to the significant savings in time and money, rapid biomass analysis methods can be used to provide levels of information that were not previously available by allowing investigations, like genetic screening or feedstock assessment that require compositional analysis of hundreds or even thousands of samples. These studies would have been too costly to pursue without the savings in time and cost provided by rapid biomass analysis methods. The development and demonstrated use of rapid analysis methods for the chemical characterization of biomass feedstocks, liquid, solid and slurry samples of pretreated biomass will be presented.

In support of the anticipated needs of an emerging biomass conversion industry, the following work plan is proposed. Wet chemical methods for biomass feedstock and biomass-derived materials be improved, validated and published as standard methods. Rapid analysis methods are being developed using a variety of spectroscopic techniques, spectrometers and multivariate analysis software packages. Procedures are being developed for the standardization of spectra collected on different instruments and for the accurate transfer of PLS equations between instruments and software packages. Demonstration methods are already being used at NREL to support ongoing research in the optimization of dilute acid pretreatment of corn stover feedstock for conversion into liquid fuels and chemicals. These methods are currently laboratory based, but plans have been made to develop at-line and on-line versions for demonstration in the DOE/NREL pilot plant.

The major challenge in the use of rapid biomass analysis is the cost of method development, estimated to be around $300,000 per method. Since these methods are feedstock and process specific, several methods may need to be developed to adequately monitor a process and core

calibration must be customized for each process environment. One proposed solution to this challenge is the organization of an industry-wide rapid analysis network where the cost of development and validation of core methods is shared. More information on a proposed Biomass Rapid Analysis Network, BRAN, is presented in the Industrial Partnership section.

Introduction

The ability to obtain an accurate chemical composition of biomass and biomass-derived samples using rapid and inexpensive methods is an important enabling technology for the commercialization of processes that convert biomass to bio-based materials, power, fuels and chemicals. The goal of this work is to develop modern analytical tools specifically for the compositional analysis of biomass and biomass-derived materials. Rapid biomass methods are being developed that offer many advantages including low per sample cost, real-time data, at-line measurement, an field-mobile measurements.

Background

Traditional Wet chemical Methods for Biomass Compositional Analysis

For more than 25 years the National Renewable Energy Lab has maintained an area of excellence in the analysis of biomass feedstocks and biomass-derived materials. Over the years, researchers and analysts at NREL have worked with a wide variety of biomass samples including hardwoods, softwoods, grasses, agricultural residues and materials produced from the conversion of biomass into power, liquid fuels, valuable chemicals and bio-based materials. Robust methods for biomass analysis have been written at NREL, validated in collaboration with the International Energy Agency and published through the American Society for Testing and Materials, ASTM. Standard biomass reference materials, prepared at NREL are available through the National Institute of Standards Technology, NIST. The ASTM methods and NIST standard reference materials can be used as a QA/QC pair to monitor data precision and accuracy and to document the quality of biomass analytical data. These traditional wet chemical methods of analysis, though robust and accurate, are not useful in a large-scale biorefinery setting because they are very expensive (labor intensive) and cannot provide the analysis information in a time frame useful for process control. For example, a complete analysis using standard wet chemical methods costs $800 - $2,000 per sample and results are not available for days, sometimes weeks.

New Tools for Biomass Compositional Analysis

To adequately serve the needs of the emerging bioenergy industry, new biomass analysis tools are needed that can perform the same analysis for about $10 per sample. These new biomass analysis methods must provide compositional data in minutes, a time frame rapid enough to guide the process adjustments necessary for steady-state production. Our approach to reducing the time and cost of compositional analysis is the development of rapid analysis methods, where multivariate analysis techniques are used to extract chemical information from easily obtained spectroscopic data. Rapid analysis methods often match the precision and accuracy of their calibration methods, so the advantages are obtained without loss of precision or accuracy (1). New techniques, like rapid analysis, are needed to provide analytical support for large-scale processes that convert biomass feedstock into fuels, chemicals, power and advanced materials.

Need for Compositional Analysis in Biomass Conversion Processes

Robust analytical methods are needed to support and enable biomass conversion processes because of the compositional variability that is an inherent property of biomass. The chemical

composition of a biomass feedstock varies as a function of many factors including plant genetics, growth environment, harvesting method, and storage. (2). Many biomass feedstocks are residues of another process, which introduces an additional source of compositional variance. For example, the users of sugar cane bagasse as a conversion feedstock must deal with the natural variance of the sugar cane plus the variance imposed by the efficiency of the sugar production process. All of these sources of compositional variance are difficult to control and their cumulative effect on the variance in feedstock composition is potentially large enough to influence process profitability (2). In addition to significant savings in time and money, rapid analysis methods can be used to provide levels of information that were not previously available. For example, feedstock variability assessments storage studies and genetic studies require the screening of hundreds, sometimes thousands of samples. Such studies would be too costly to pursue without the savings in time and cost provided by rapid analysis methods.
The ability to accurately sample a bulk feedstock and to analyze hundreds of samples for about $10 each provides a new tool that is being used at NREL to assess the compositional variability of corn stover in the U.S. as a function of variety, geographical location, harvest time and collection method. Changes in feedstock composition near the time of harvest and during storage are also being monitored. As will be presented later, significant differences have already been seen in the composition of bulk corn stover feedstocks.

Application of Rapid Biomass Analysis in an Industrial Process

As shown in Figure 1, rapid biomass analysis can be useful at many stages of an industrial process. If necessary, rapid analysis can be used to guide feedstock blending to provide a more uniform feedstock composition. The composition of a feedstock can be measured as it enters the conversion reactor, and chemical changes during the processing of biomass can be monitored to provide feed-forward and feed-backwards information that can be used to ensure that the process remains efficient in spite of the feedstock variability. As more samples are analyzed, information can be obtained about the composition of an "ideal feedstock". Field- mobile instruments can be calibrated for use as purchasing tools. Buyers can obtain compositional information about a biomass feedstock at the point of purchase. Feedstock prices can then be based on quality instead of weight. Living plants, young plants and perhaps even seeds can be evaluated and selected for desirable characteristics and production potential. The rapid, inexpensive compositional analysis methods described here are examples of new tools that support the commercialization of biomass conversion technologies by providing the information necessary to accommodate biomass variability.

The depth of experience in biomass characterization and multivariate analysis along with broad research being conducted in many areas of biomass conversion made NREL a natural site for the development of rapid biomass analysis tools. The research environment, which includes both fundamental and applied projects, provides an abundance of diverse biomass samples to be used for method calibration. The pilot-scale reactors provide an ideal setting for developing, validating and demonstrating the use of rapid biomass analysis methods. Imput from Instrument manufacturers, feedstock providers and industrial partners guides development priorities and promotes the development of the most relevant and necessary tools.

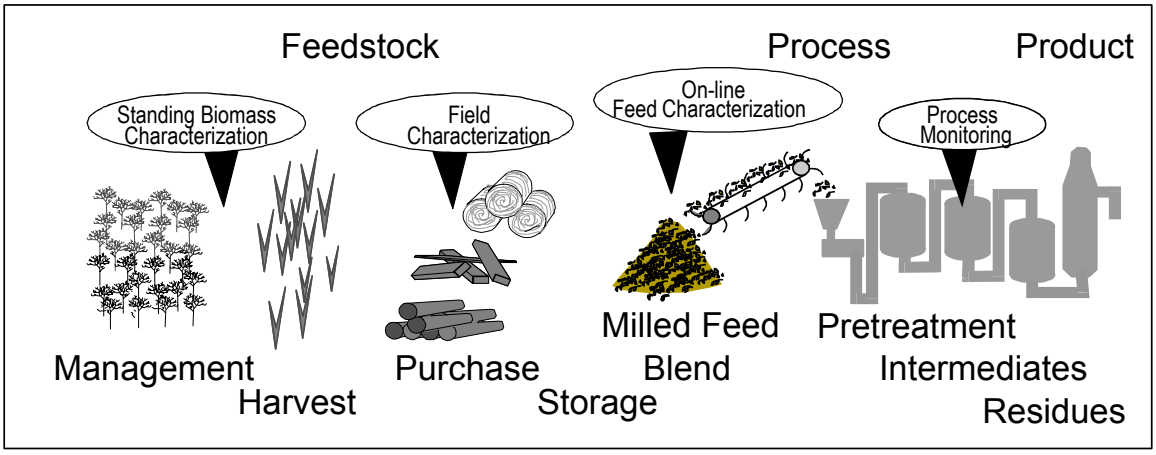

Figure 1. Applications for Rapid Biomass Analysis in Biomass Conversion Processes

Rapid Analysis Method Development

Several steps are involved in the development of rapid biomass analysis methods, including gathering appropriate calibration samples, chemical characterization of the calibration samples, developing spectroscopic methods for the rapid technique, PLS regression and the validation of the PLS algorithm and the development of QA/QC procedures including guidelines for appropriate application of the new methods.

Calibration Samples

The first step in developing a new method is the gathering of appropriate calibration samples. A minimum of 30 unique samples is needed for preliminary methods. Calibration sets for robust methods usually contain 100-300 well-characterized samples. Rapid biomass analysis methods require accurate compositional data for all calibration samples. Rapid analysis methods look for patterns in spectroscopic data that correlate with changes in composition. Strong correlations will not be found when calibration compositional data is of poor quality. Since the rapid analysis method will retain the precision and accuracy of the calibration data, the calibration data should be obtained using the most robust and accurate methods available. Calibration samples should have compositions similar to the samples to be analyzed and samples should reflect all known sources of compositional variance for that material. Another feature that is essential to a good

calibration set is independent variance of the different constituents. Research facilities where process parameters are intentionally varied are the ideal environment for collecting calibration samples with the required diversity. A diverse calibration set with quality calibration data is required for an accurate and robust rapid analysis method. Collecting and characterizing a good calibration set costs around $300,000, which is by far the most expensive and time-consuming step in method development.

Spectroscopic Methods

Quality spectroscopy is the second essential component of method development. Although many different spectroscopic techniques can be used in the development of rapid analysis methods, near infrared (NIR) spectroscopy offers several advantages for biomass analysis. A wide variety of instruments are commercially available including rugged NIR spectrometers designed for use outdoors or for process monitoring (3). Sample handling devices have been developed specifically for the analysis of bulk biomass samples and biomass-derived liquids and slurries. Near infrared spectrometers collect data from the visible and NIR regions of the electromagnetic spectrum from 400 to 2500 nm. This region contains signals from C-H, O-H, N-H and C=O bonds, so compositional information for cellulose, hemicellulose, protein and lignin is contained in the NIR spectra of biomass samples. For biomass solids, NIR spectroscopy is usually done in reflectance mode. This is a surface technique capable of penetrating only about 150 microns into the biomass surface. To obtain a representative spectrum of biomass, samples must be ground to a uniform particle size or a large sample volume must be scanned during spectral collection. In most cases 30-100 spectra are collected and averaged prior to PLS analysis. Reflectance spectroscopy separates the detector from the NIR light source allowing sunlight to be used as the NIR source in field measurements. The spectroscopic technique selected must contain the desired information about the chemical composition of each sample. Since inorganic materials do not absorb infrared light, non-structural materials like soil cannot be measured using near infrared wavelengths. Structural inorganics can be measured indirectly if they perturb the absorbance of adjacent organic compounds. Figure 2 shows the NIR spectra of nine corn stover anatomical fractions. These spectra contain all of the necessary information to determine the chemical compositional of these samples. The selection of an appropriate spectroscopic method is the key to cost reduction and speed of analysis. The final rapid analysis method will extract chemical information from spectroscopic patterns. Once calibration is complete, compositional analysis becomes as fast and inexpensive as the spectroscopic method.

Multivariate Analysis

Multivariate analysis connects the compositional data with the spectroscopic data. The projection to latent structures (PLS, sometimes called partial least squares) method regresses the matrix of compositional information against NIR spectral data. In simplified terms, PLS analysis solves hundreds of equations in thousands of variables to obtain a linear equation that translates spectroscopic data into compositional information. Multivariate analysis methods, like PLS regression, were designed for complex systems like those found in biomass compositional analysis. Rapid analysis techniques using NIR spectroscopy are common in industrial applications monitoring the composition of animal feed, cheese, beer and grain. The concept of NIR/PLS analysis of biomass is not novel. Only the application of this proven technology to

biomass conversion and the development of calibrations specific for energy feedstocks is new. Method development in this area has great potential for high impact with low risk.

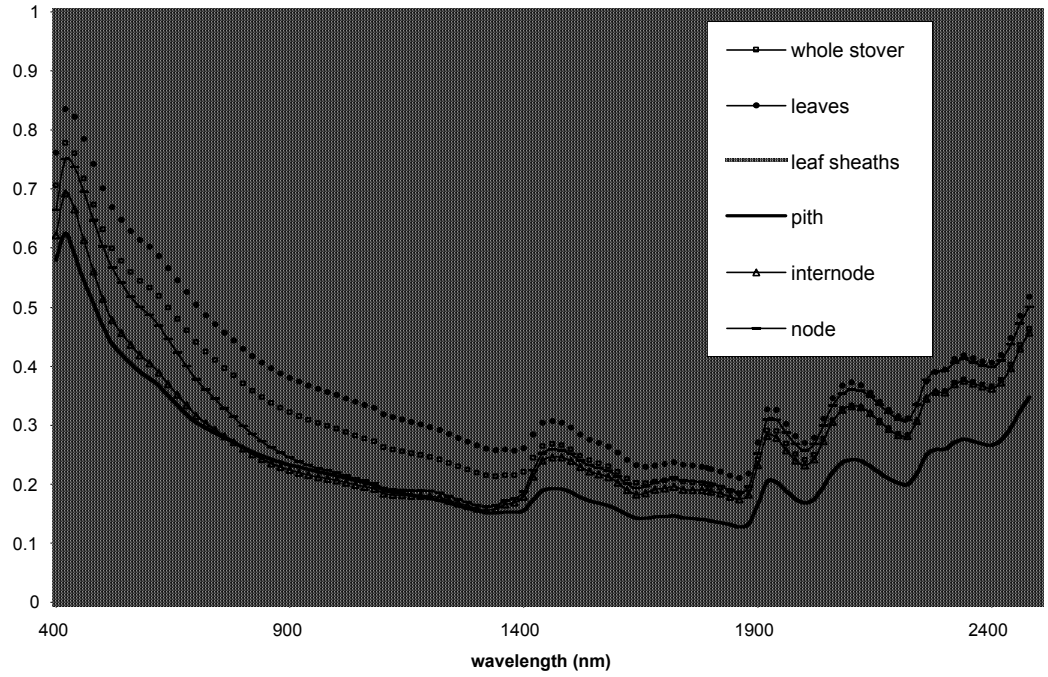

Figure 2. Near Infrared Spectra of Anatomical Fractions of Corn Stover

Quality Assurance and Quality Control

In addition to the rigorous QA/QC procedures required in obtaining the compositional data for the calibration samples, special safeguards should be established to assure that the rapid methods are appropriately applied to the analysis of unknown samples. QA/QC tests should first determine that the unknown samples are similar in composition to the samples used in the method calibration. Most multivariate analysis software packages include principal component analysis methods that can be used for this purpose. Spectroscopic methods should be standardized, particularly in optical configurations and pathlength and the same spectroscopic methods should be used for both calibration samples and unknown samples. Multivariate analysis methods should be tested using full cross validation techniques and test using blind samples. Implementing rigorous QA/QC procedures during method development will facilitate calibration sharing and calibration transfer.

Rapid Biomass Analysis: Supporting NREL Research

Corn stover has been selected as a model feedstock for its Enzyme Sugar Platform (ESP) work. In support of ESP work, NREL has developed several laboratory-based rapid analysis methods

for the compositional analysis of corn stover feedstocks and corn stover-derived intermediates from a dilute acid pretreatment process. The corn stover feedstock model uses NIR reflectance techniques similar to those that are widely used in feed and forage analysis. Work continues on improving the accuracy of the feedstock model for minor constituents and to expand the calibration range for all constituents. Three process intermediate methods are currently being developed and improved, a NIR reflectance method for the analysis of dry solids, a transmission spectroscopy method for the analysis of filtered hydrolysis liquors and a transflectance spectroscopy method that allows compositional information to be obtained directly from wet process slurries. Field methods are also being developed that measure the composition of growing corn plants. These methods support genetic screening studies and feedstock engineering.

Feedstock NIR/PLS Method

The calibration set for the compositional analysis of corn stover feedstocks contains 54 samples representing five locations and three harvest years. The calibration set includes aged stover samples and hand-separated anatomical fractions. The accuracy of the NIR/PLS method is illustrated in Figure 3, which compares the composition determined by wet chemistry to that estimated by the NIR/PLS equation. The diagonal line represents perfect agreement. The NIR/PLS data are from a full cross validation model, which provides a conservative estimate of the accuracy of the final equation. The distribution of points parallel to the line represents the calibration range. The distribution of the points perpendicular to the line represents error in the NIR/PLS method. The graph demonstrates that the NIR/PLS methods provides a complete compositional analysis for a wide range of samples with precision and accuracy that match the wet chemical methods used to obtain the calibration data.

Application of Feedstock Method

The ability to accurately sample a bulk feedstock and to analyze hundreds of samples for about $10 each provides a new tool that is being used at NREL to analyze more feedstock samples in an expanded effort to assess the compositional variability in bulk corn stover used in our in-house research. Significant differences have already been seen in feedstock composition, which appear to be reflected in all stages of ethanol production. Figure 4 compares compositional information for three samples from the 2000 harvest and two from the 2001 harvest. The potential value of these bulk feedstocks for ethanol production is indicated by a theoretical maximum ethanol yield. The theoretical maximum yield assumes that 100% of the sugars are converted to ethanol. This value can be calculated from the carbohydrate content (4). The theoretical maximum ethanol yields for these feedstocks vary from 105 gallons per dry ton to 119 gallons per dry ton. Clearly, weight alone is not a good measure of feedstock value. The differences shown in carbohydrates, lignin, inorganics and acetyl content are of a magnitude that could significantly impact process economics. Compositional studies are underway to improve our understanding of corn stover feedstock value and compositional variance as a function of location, genetics, and cultivation, harvest and storage methods. Changes in feedstock composition around the time of harvest and during storage are also being monitored in support of projects funded through Oak Ridge National Laboratory. The results of these studies will be presented later in a separate section on feedstock variability.

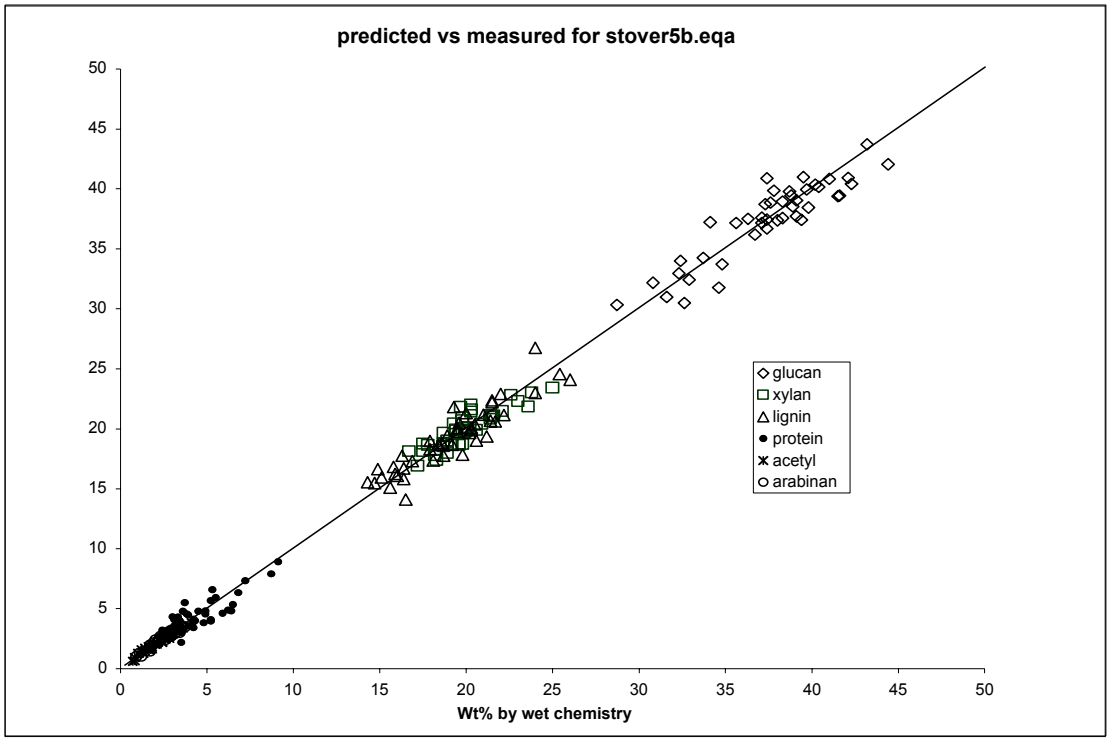

Figure 3. Comparison of Corns Stover Feedstock Composition as Determined by Wet Chemical and NIR/PLS Methods

The NIR/PLS rapid analysis method for feedstock characterization is also being used to screen thousands of corn plants to identify interesting cell wall mutations. One such study quickly selected 44 individual plants from a population of 2,000 for further DNA analysis (not shown). Each of these individuals had a xylan content that was significantly different from the general population. These individuals belonged to 11 families and represented the expected 1/4 ratio of an otherwise normal family. No unusual compositions were reported for members of the four inbred families used as negative controls. The rapid analysis method provided a means of selecting unusual samples based on their unusual cell wall chemistry.

Field-based methods for the analysis of live plants are in the early development stages. These methods are being developed for use in large-scale genetic screening projects and will eliminate the costly and time-consuming milling and drying steps. The ability to identify interesting plants before flowering may improve the efficiency of cross breeding experiments.

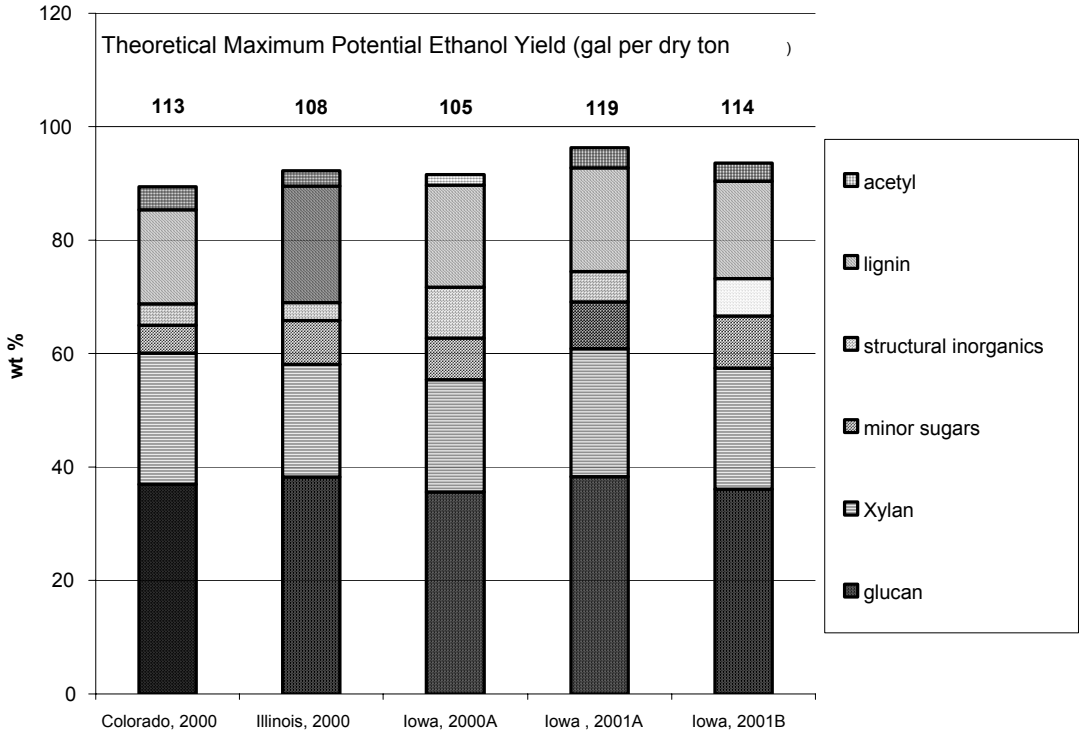

Figure 4. Comparison of Theoretical Ethanol Yields and Composition of Bulk Corn Stover Feedstocks

Process Intermediate Methods

Dry Pretreated Solids Method. The calibration set for determining the chemical composition of dry process intermediates solids from the acid pretreatment of corn stover consists of 98 samples obtained from experiments that used four feedstocks, and a variety of pretreatment reactors operated at varying severities. The accuracy of the NIR/PLS method is illustrated in Figure 5, which compares the composition determined by wet chemistry to cross validation estimates of the composition using the NIR/PLS equation. The diagonal line represents perfect agreement. The graph demonstrates that the NIR/PLS methods provides a complete compositional analysis for a wide range of samples with precision and accuracy that match the wet chemical methods used to obtain the calibration data.

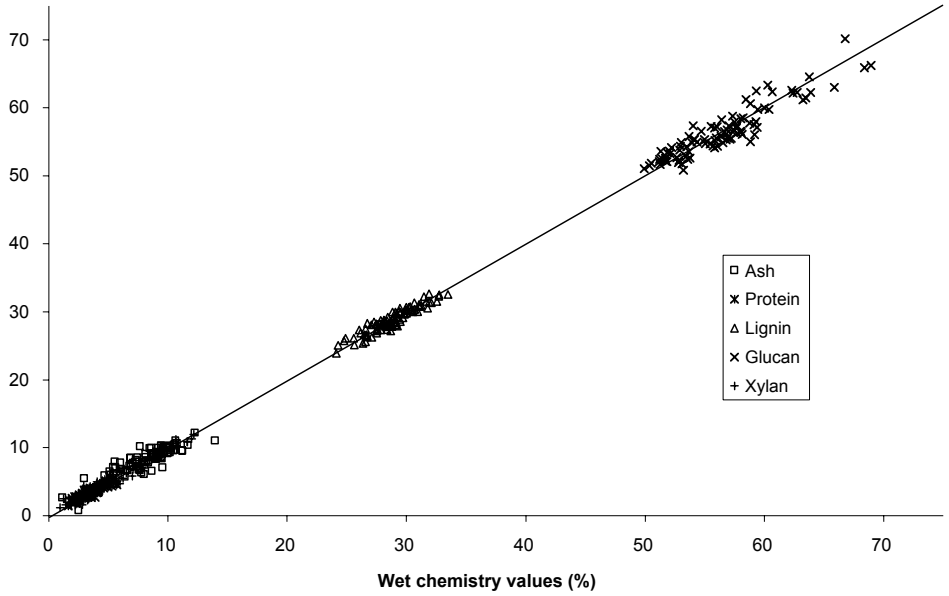

Figure 5. Comparison of "true" composition as determined by standard wet chemical methods and the cross validation composition as predicted by NIR/PLS methods using stovint5.eqa.

Slurry Process Intermediate Method (preliminary method). The calibration set for determining the chemical composition of wet process intermediate solids from the acid pretreatment of corn stover consists of 16 samples obtained from experiments that used three feedstocks and different pretreatment reactors operated at varying severities. This rapid analysis method was calibrated using as-received slurried, pretreated solids that were scanned by NIR as received, using the supernatant liquor from the slurry as the reference blank. The constituent values used in the method calibration were obtained from the isolated, dried solids as described in the dry solids method. The accuracy of the slurry NIR/PLS method is illustrated in Figure 6, which shows the excellent correlation between the cross validation prediction and the "true" chemical composition of the calibration samples. The range of calibration for each constituent can be seen. The spread perpendicular to the line of perfect fit reflects the errors in the wet chemical calibration methods. Figure 6 also shows compositions of the same pretreated solids as predicted using the dry solids method described above. The agreement between compositions predicted from dry samples and composition predicted from wet samples is demonstrated. This suggests that many sample preparation steps could be eliminated without impacting data quality. The ability to obtain a complete chemical characterization on wet pretreatment solids in a process slurry moves rapid analysis one step closer to the goal of on-line and at-line measurements.

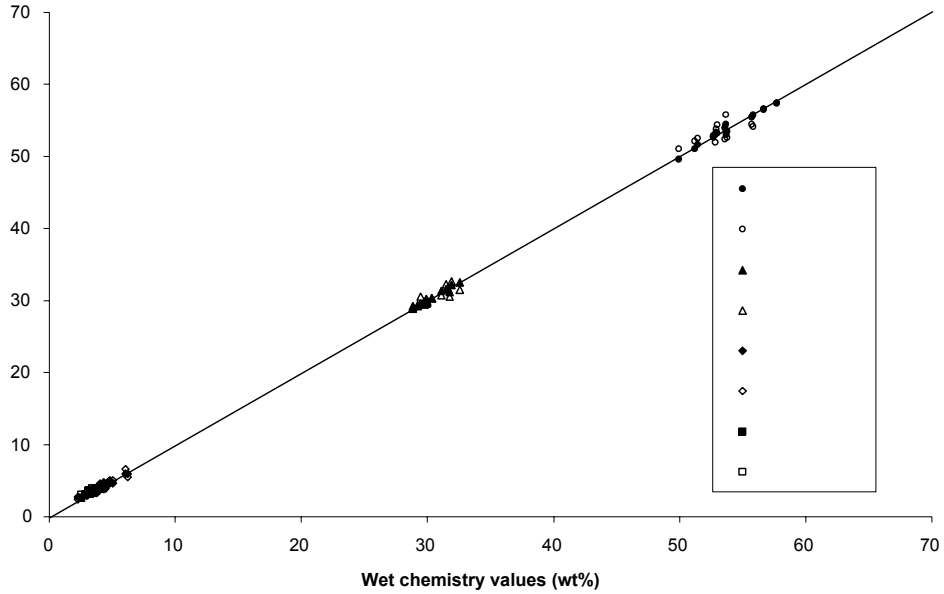

Figure 6. Comparison of "true" composition as determined by standard wet chemical methods, the composition as predicted from dry solids using stovint5.eqa and the composition as predicted from wet solids using slurry1.eqa. Predicted values are from cross validation.

Liquid Process Intermediate Method (preliminary method). This NIR/PLS rapid biomass analysis method was developed to determine the chemical composition of biomass-derived materials dissolved in the pretreatment liquor during dilute acid pretreatment of corn stover. The calibration set for this method consists of 29 pretreatment liquor samples obtained from experiments that used three feedstocks and different pretreatment reactors operated at varying severities. This rapid analysis method was calibrated using NIR transmission spectra collected on liquor samples obtained from pretreatment corn stover slurries after filtration to remove all suspended solids. Eighteen parameters were measured on the filtered liquids and used in the PLS method calibration.

For ease of viewing, the predicted vs. measured graphs for this method have been split into four separate charts. Figure 7 shows the correlation between the cross validation prediction and the "true" chemical composition for major sugars in the calibration samples. Figure 8 shows the correlation between the cross validation prediction and the "true" chemical composition for minor sugars in the calibration samples. Figure 9 shows the correlation between the cross validation prediction and the "true" chemical composition for glycerol, lactic acid and pH in the calibration samples. Figure 10 shows the correlation between the cross validation prediction and the "true" chemical composition for acetyl, lignin HMF, and furfural in the calibration samples. The range of calibration for each constituent can be seen. The spread perpendicular to the line of perfect fit reflects the errors in the wet chemical calibration methods. The ability to obtain a complete chemical characterization directly from filtered pretreatment liquors moves rapid biomass analysis another step closer to the goal of on-line and at-line measurements.

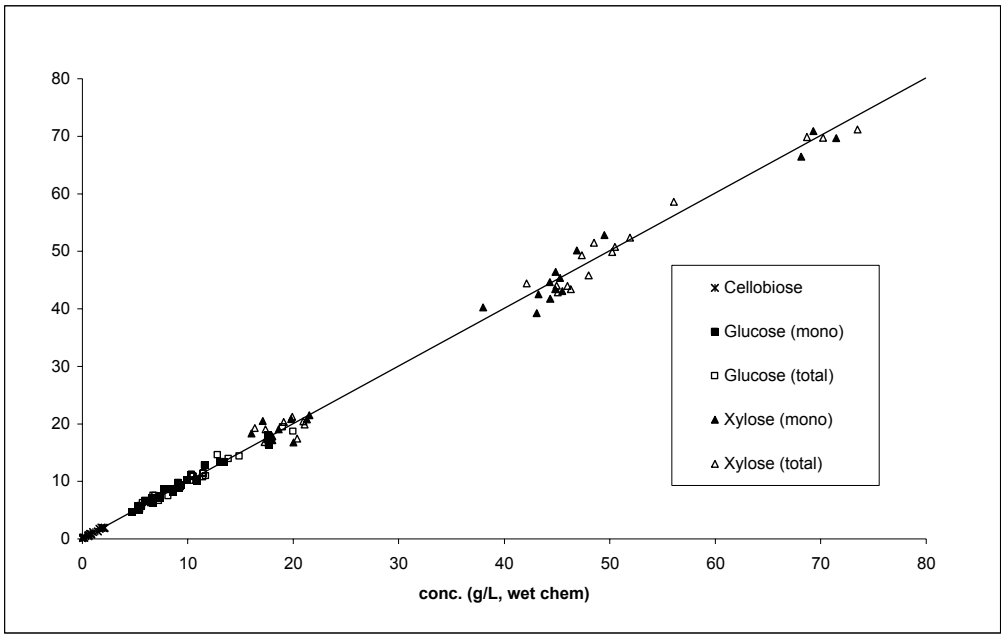

Figure 7. Comparison of "true" composition as determined by standard wet chemical methods and the composition as predicted by NIR/PLS methods for major sugars using liquors1.eqa.

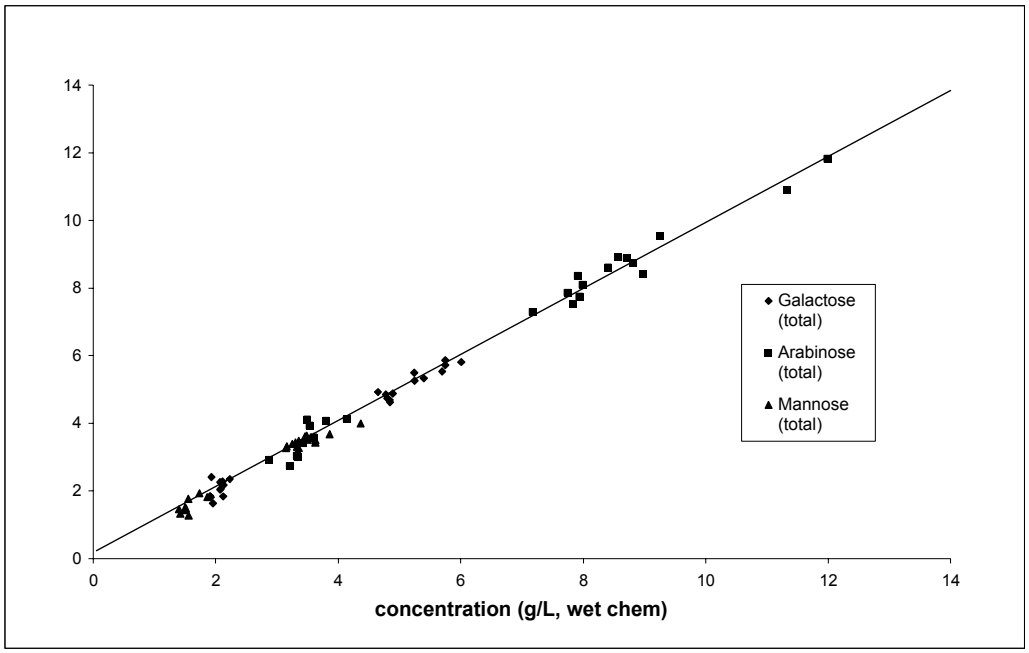

Figure 8. Comparison of "true" composition as determined by standard wet chemical methods and the composition as predicted by NIR/PLS methods for minor sugars using liquors1.eqa.

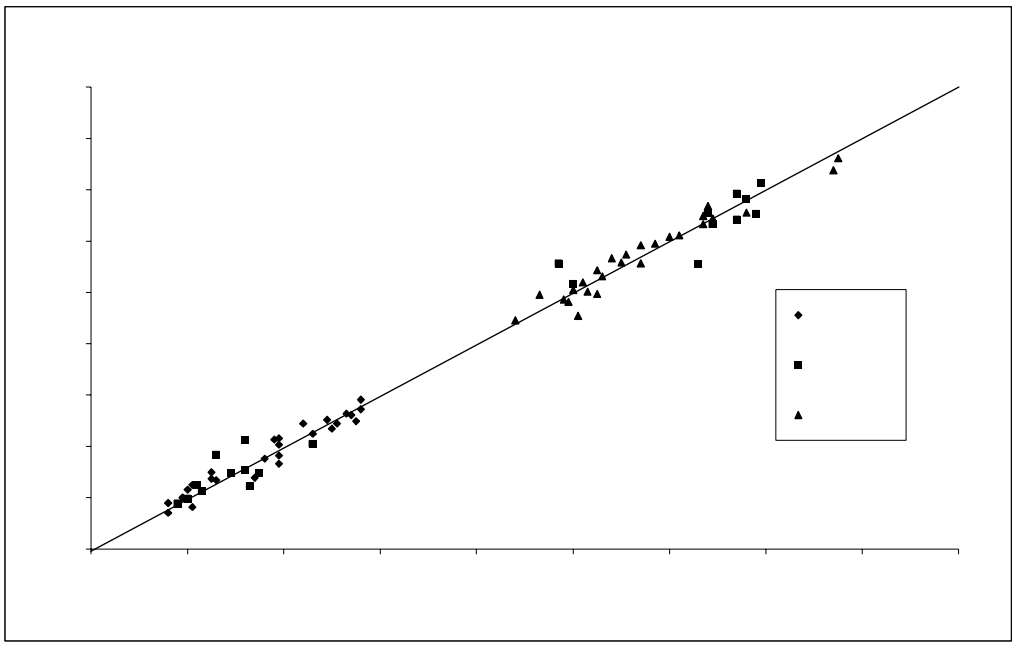

Figure 9. Comparison of "true" composition as determined by standard wet chemical methods and the composition as predicted by NIR/PLS methods of glycerol, lactic acid and pH using liquors1.eqa.

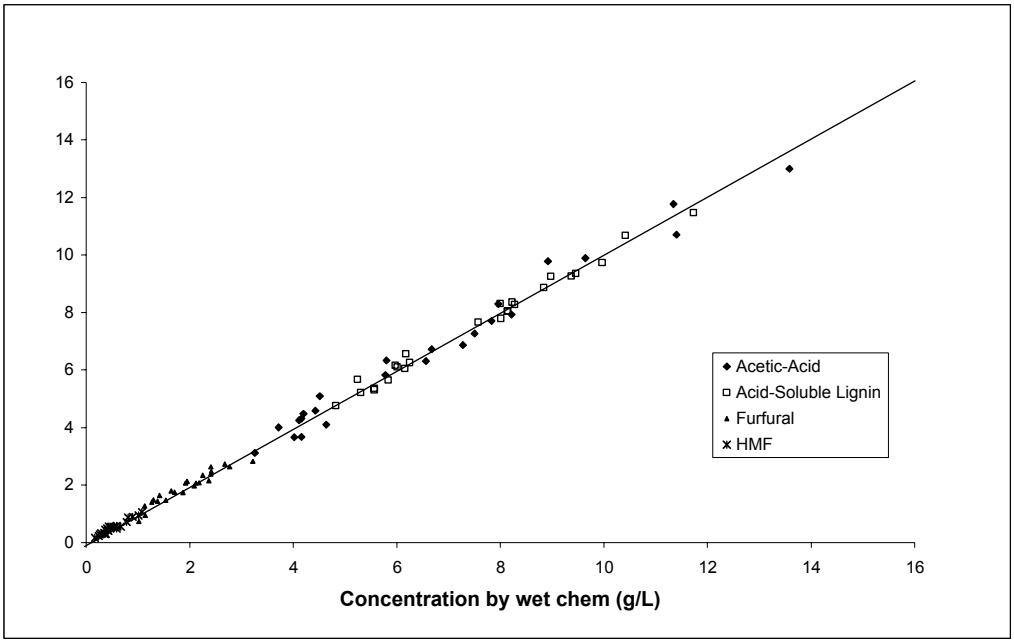

Figure 10. Comparison of "true" composition as determined by standard wet chemical methods and the composition as predicted by NIR/PLS methods of acetic acid, lignin, HMF and furfural using liquors1.eqa.

Application of the Process Intermediate Methods

This NIR/PLS rapid biomass analysis method is being used to evaluate the performance of dilute acid pretreatment of corn stover. Figure 11 shows compositional differences in pretreated samples of corn stover as measured by NIR/PLS on dried pretreated stove solids. Rapid analysis allows processes operators to determine when the sample is depleted in xylan and is ready for the next process step, enzymatic hydrolysis. Stopping pretreatment early will leave hemicellulose in the sample, which decreases enzyme efficiency (5). Continuing pretreatment beyond the point of xylan removal will result in the loss of fermentable sugars, which lowers potential ethanol yields. In a similar manner, the liquid NIR/PLS method could be used to optimize the pretreatment process by monitoring oligomer-to-monomer hydrolysis and at the same time monitor monomer degradation.

When used together, the feedstock and process intermediate methods can enhance our fundamental understanding of the pretreatment process. Compositional data can be used as a feed-forward or feed backward tool for process control and optimization.

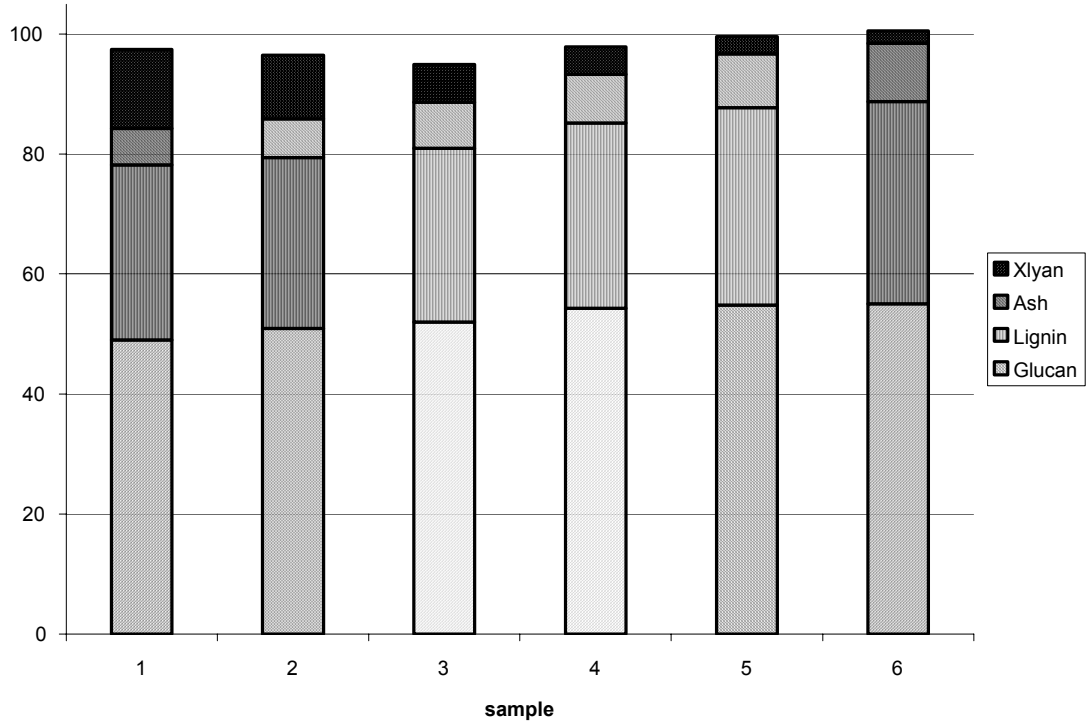

Figure 11. Composition of Pretreated Corn Stover Samples Demonstrating Optimization of Pretreatment for Xylose Removal

Research Plans and Directions

In support of the anticipated needs of an emerging biomass conversion industry we propose continued work in the following areas:

1. Wet chemical methods for biomass feedstock and biomass-derived materials are being improved, validated and published as standard methods. Since the rapid analysis methods retain the precision and accuracy of the wet chemical methods, improving the calibration methods is necessary for improved rapid methods. New wet chemical methods need to be validated and QA/QC protocols established before the methods can be published as standard consensus methods. Publication of the validated standard methods provides the industry with a common ground for comparison of various feedstocks and conversion processes.

2. Rapid analysis methods are being developed using a variety of spectroscopic techniques, spectrometers and multivariate analysis software packages. Procedures are being developed for the standardization of spectra collected on different instruments and for the accurate transfer of PLS equations between instruments and software packages. Validation of core rapid analysis methods can be done at the point of method development or across and industrial group. Deployment of these methods to industrial

partners will require customization and validation in the new setting. Understanding the challenges associated with calibration transfer is key to successful technology transfer.

3. Demonstration methods are already being used at NREL to support ongoing research in the optimization of dilute acid pretreatment of corn stover feedstock for conversion into liquid fuels and chemicals. These corn stover rapid analysis methods are currently laboratory based, but plans have already been made to develop at-line and on-line versions for demonstration in the NREL pilot development plant. Deployment and testing of rapid biomass analysis methods at the pilot scale should provide valuable information about the challenges associated with on-line measurement. At the same time information will be obtained about the use of these methods to optimize biomass conversion processes in real-time.

Biomass Rapid Analysis Network

The major challenge in the use of rapid biomass analysis is the cost of method development, estimated to be around $300,000 per method. Since these methods are feedstock and process specific, several methods may need to be developed to adequately monitor a process and core calibration must be customized for each process environment. One proposed solution to this challenge is the organization of an industry-wide rapid analysis network where the cost of method development is shared. Core methods could be developed, validated and published as standard consensus methods. This network could facilitate communication for troubleshooting and problem solving and provide training opportunities in wet chemical calibration methods, advanced spectroscopic techniques, multivariate analysis, QA/QC procedures and long-term method maintenance. Participating members could look to the network for the latest information on instruments, multivariate analysis software, calibration transfer and calibration customization. More information on a proposed Biomass Rapid Analysis Network, BRAN will be presented in the Industrial Partnership section.

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